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Effects of Combined Treatments on the Radiosensitivity of *Aspergillus flavus* and *Penicillium islandicum*

by

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Aspergillus flavus 및 *Penicillium islandicum* 의 放射線 感受性에 미치는 倂用처리의 效果

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Abstract

Effects of combined treatments of radiation-heat and radiation-chemicals on the survival of mycotoxin-producing molds *Aspergillus flavus* and *Penicillum islandicum* conidia were investigated.

Combined treatments of gamma-radiation and heat (10 minutes at 50°C or 55°C) showed a synergistic effect, causing remarkable decreases in D_{10} values and induction doses in the two molds. Combined treatments of gamma-radiation and chemicals (sorbic acid) had no synergistic effect. *Asp. flavus* was more resistant to heat and sorbic acid than was *Pen. islandicum*.

Introduction

The importance and objective of studies on the radiosensitivity of mycotoxin-producing molds were pointed out in a previous paper[1]. Thus the effect of gamma irradiation on the survival of seven representative *Aspergillus* and *Penicillium* species isolated from deteriorated rice in Korea was investigated to obtain necessary lethal doses.

It has been known that radiosensitivity of microorganisms varies with the condition of irradiation[2-5]. Thus the effects of combined treatments of radiation and heat were studied in regard to heat-resistant bacteria in foods[5-9] and molds in fruits[10-12]. Effects of combined treatments of radiation and chemicals were also reported in view of radiosensitization of microorganisms, thus preventing the microbial spoilage of foods[13-19].

This paper describes the combined effects of gamma irradiation and heat or a chemical preservative on the survival of two representative mycotoxin-producing molds, *Aspergillus flavus* and *Penicillium islandicum* isolated from deteriorated rice in Korea. This sort of study should provide the basic data to interprete the killing mechanism and the synergistic effect in radiosensitivity of microorganisms as well as to provide the way of sterilization under such a mild condition as low irradiation dose, low temperature or low concentration of preservatives.

Materials and Methods

1. Microorganisms

Aspergillus flavus var. *columnaris* and *Penicillium islandicum* were strains isolated from deteriorated rice and handled as described in a previous paper[1].

2. Combined treatments of heat and radiation

A 2-ml aliquot of conidium suspension in a 1.2×13 cm test tube was heated for 10 minutes in a shaking water bath maintained at 50°C or 55°C and cooled rapidly in ice water. The heated spore suspension was then irradiated with gamma-ray at a dose rate of 2.67 krad/min by use of 9,000 Ci Co-60 panoramic irradiator installed at this Institute. The viable cell count was made as described in a previous paper.[1]

Heat resistance of spores was measured by holding the spore suspension for 10 minutes in a shaking water bath maintained at a definite temperature.

3. Combined treatments of radiation and chemicals

A 2-ml aliquot of spore suspension in a 1.2×13 cm test tube was irradiated with 60 krad of gamma-ray at a dose rate of 2.45 krad/min and spread on Czapek agar plates containing various concentrations of sorbic acid. The plates were incubated 4~10 days at 30°C for the viable cell count.

Results and Discussion

The effect of heat on the survival of conidia of test organisms is given in Fig. 1. *Asp. flavus* showed a stronger heat resistance than *Pen. islandicum*. That is, the conidia of *Asp. flavus* survived 86% at 50°C and 63% at 55°C while those of *Pen. islandicum* survived 69% at 50°C.

Combined effects of heat followed by radiation on the survival of two molds are shown in Fig. 2, 3 and Table 1. The effects were synergistic, causing decreases in D_{10} values and induction doses, and increases in dose multiplying factors. As an example in the case

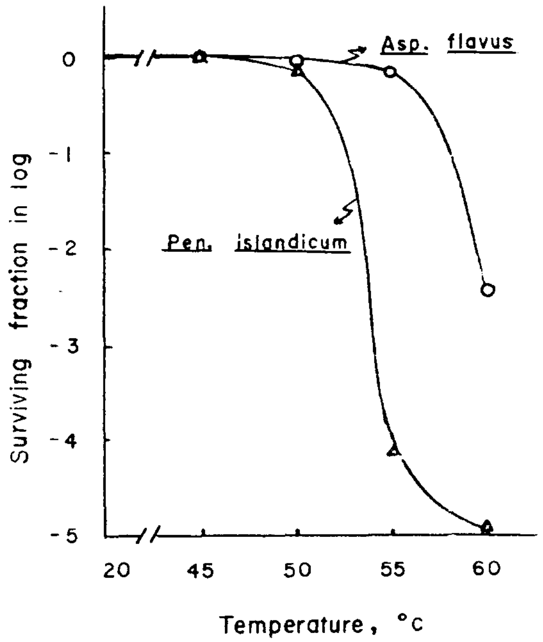

Fig. 1. Thermal stability of *Asp. flavus* and *Pen. islandicum* conidia in 10-minute heating

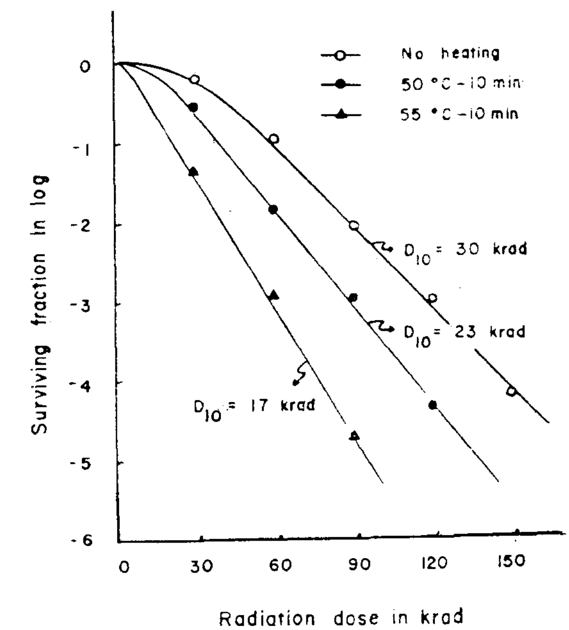

Fig. 2. Combined effects of radiation and heat on the survival of *Asp. flavus* conidia

Table 1. Effects of pre-irradiation heating on the survival of *Asp. flavus* and *Pen. islandicum* conidia*

	Asp. flavus			*Pen. islandicum*	
	No heating	50°C	55°C	No heating	50°C
Induction dose (krad)	48	24	6	42	6
D_{10} value (krad)	30	23	17	30	24
Inactivation factor at 200 krad	5.1	7.7	11.4	5.3	8.1
Dose multiplying factor**	1.00	1.30	1.76	1.00	1.25

* The conidium suspensions were heated 10 min at a definite temperature and irradiated at a dose rate of 2.67 krad/min.

** Obtained by dividing the D_{10} value of control with those of heated samples.

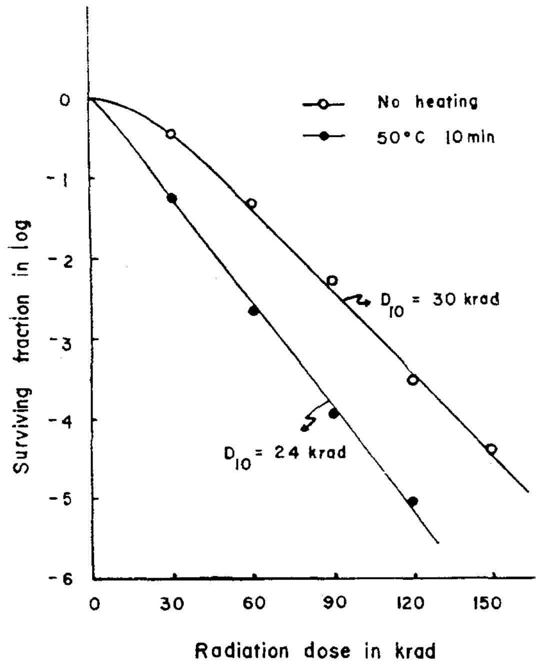

Fig. 3. Combined effects of radiation and heat on the survival of *Pen. islandicum* conidia

of *Asp. flavus*, the D_{10} value was 30 krad in radiation treatment only and it decreased to 23 and 17 krads in combined treatments of radiation and heat at 50°C and 55°C, respectively. This effect was more remarkable in induction doses which decreased from 48 krad in no-heating to 24 and 6 krads in 50°C and 55°C heating, respectively. These phenomena should be better demonstrated in terms of inactivation factor. That is, the surviving fraction of *Asp. flavus* conidia can be diminished to $10^{-11.4}$ by heating 10 minutes at 55°C followed by 200 krad irradiation while it can be decreased to $10^{-5.1}$ by 200 krad irradiation only.

The synergistic effects of radiation and heat on microorganisms may be effectively utilized in low dose irradiation for sterilization purpose and thus were investigated with particular emphasis on bacteria for a long time.[5-11] Heat treatment has been widely used as an effective way of sterilization from the early time but it has a drawback of causing physical and chemical damages in foods. Therefore, low temperatures such as 50~55°C were used in this study to prevent the denaturation of proteins and irradiaton was followed, though sterilization can not be expected at these temperatures as shown in Fig. 1. The synergistic effects between radiation and heat were reported to vary with the order of treatments. For example, post-irradiation heating was more effective for spores of the bacterium *Clostridium botulinum* while pre-irradiation heating was better for molds such as *Pullularis* and *Penicillium*.[12,20] It appears that this is due to the difference in repair mechanism of DNA in the organisms though the real cause remains to be answered.[21,22]

Combined treatments of radiation and chemicals have been attempted to study the sensitizing effect of chemicals toward irradiation. Chemicals used in connection with radiosterilization include salts of sodium, potassium, iodine and vitamin K[2,13-17,20] and they were employed to observe their effects on the reproduction of microorganisms irradiated in the presence of the chemicals. In this experiment, spore suspensions of *Asp. flavus* and *Pen. islandicum* were irradiated with 60 krad ($2\times D_{10}$ value) gamma ray and examined for their ability to survive on an agar plate containing sorbic acid. Fungicidal action of sorbic acid was two

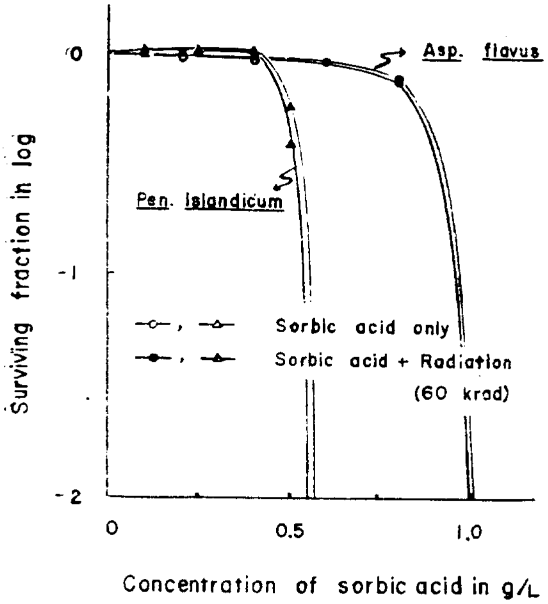

Fig. 4. Combined effects of radiation and sorbic acid on the survival of *Asp. flavus* and *Pen. islandicum* conidia

times stronger for *Pen. islandicum* than for *Asp. flavus*, which needed 1,000 ppm of sorbic acid for 99% killing. However, no synergistic effect was observed in both species as shown in Fig. 4. This result is different from others that sorbic acid in combination with radiation showed a protective effect on *Pen. expansum* and a sensitizing effect on *Escherichia coli*[23]. It was also reported[18] that the storage period of cheese irradiated at 100~200 krad was extended by packing with sorbic acid-treated cellophane. It appears that the synergistic effects between radiation and chemicals vary with microbial species and further studies are needed to make the practical application of the combination treatments in food preservation practices possible.

요 약

Mycotoxin 生成菌인 *Aspergillus flavus* 와 *Penicillium islandicum* 分生胞子의 生存에 미치는 방사선과 가열 또는 약품의 併用처리효과를 실험하여 다음과 같은 결과를 얻었다.

감마선과 가열(50°C 또는 55°C 에서 10분 처리)의 병용처리는 두 곰팡이에서 현저한 相乘効果를 보여 D_{10}값과 誘導線量이 크게 감소하였으나 방사선과 약품(sorbic acid)의 병용처리는 아무런 상승효과도 보이지 아니하였다. *Asp. flavus* 는 *Pen. islandicum* 에 비하여 가열 및 sorbic acid에 대한 저항성이 더 컸다.

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